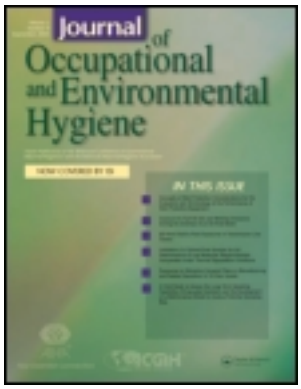

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An Investigation into Techniques for Cleaning Mold-Contaminated Home Contents

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An Investigation into Techniques for Cleaning Mold-Contaminated Home Contents

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*This study examined the efficacy of the following treatments to reduce selected fungal spore and mycotoxin levels on materials commonly found in home contents: (1) gamma irradiation at a 10–13 kiloGray exposure, (2) a detergent/bleach wash, and (3) a steam cleaning technique. A minimum of six replicates were performed per treatment. Paper, cloth, wood, and carpet were inoculated with either fungal spores (*Stachybotrys chartarum, Aspergillus niger, Penicillium chrysogenum*, or* Chaetomium globosum*) at 240,000 spores/2.54 cm^2 of material or with the mycotoxins roridin A, T-2, and verrucarin A at 10 μg per 2.54 cm^2 of material. Treatments were evaluated with an agar plating technique for fungal spores and a yeast toxicity culture assay for mycotoxins. Results showed that gamma irradiation inactivated fungal spores, but the treatment was not successful in inactivating mycotoxins. The washing technique completely inactivated or removed spores on all materials except for* C. globosum*, which was reduced on all items except paper ($p < 0.05$). Washing inactivated all mycotoxins on paper and cloth but not on carpet or untreated wood ($p < 0.001$). The steam cleaning treatment did not completely eliminate any fungal spores; however, it reduced* P. chrysogenum *numbers on all materials,* C. globosum *was reduced on wood and carpet, and* S. chartarum *was reduced on wood ($p < 0.05$). Steam cleaning was unsuccessful in inactivating any of the tested mycotoxins. These results show that the bleach/detergent washing technique was more effective overall in reducing spore and mycotoxin levels than gamma irradiation or steam cleaning. However, the other examined techniques were successful in varying degrees.*

Keywords contents, fungi, mycotoxin, sick building syndrome, sterilization

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There is a great deal of confusion and misinformation both on the Internet and in other media regarding the status of household contents in mold-contaminated structures. This is because there are no studies that show a relationship between items from a mold-contaminated structure and human health. Additionally, there are no accurate and comprehensive testing methodologies for determining what levels of spores and/or mycotoxins are present on contents inside structures. For example, the identification of fungal colonies on contents can be performed using tape lift and swab sampling;[1] however, some items may have spores hidden inside them or in hard to reach areas, and these sampling techniques are often inadequate to extract the spores. Sampling techniques for spores and hyphae on contents are therefore best viewed as being approximate and not comprehensive representations.

Despite the limitations in determining the extent of fungal contamination on contents, and the lack of knowledge regarding the relationship between levels of fungal spores and/or mycotoxins and human ill-health, there is a requirement for a solution regarding the appropriate treatment of contents in a mold-contaminated dwelling. The purpose of this study was to investigate some sterilizing procedures that may be effective in eliminating fungal spores and mycotoxins on home contents.

MATERIALS AND METHODS

Representative materials of household contents used in the study were wood, cloth, paper, and carpet. Fungal spores or mycotoxins were inoculated separately onto the materials. Three techniques were used for cleaning/sterilizing the materials: (1) gamma irradiation, (2) a washing technique using a commercially available detergent/bleach combination, and (3) a commercial steam cleaner.

Materials

The paper was general purpose printing paper (Hp multipurpose paper; Hewlett Packard Co., Palo Alto, Calif.). The cloth was a white 65% cotton/35% polyester material (No. 556092; Landau Uniforms, Memphis, Tenn.). The wood was untreated 5-ply plywood, 63 mm thick. The carpet was a white 100% nylon pile with a woven polypropylene backing (Williamsville 06600 Color 127 fleece; Lowe's Home Improvement Center,

Lubbock, Texas). The materials for sampling were cut into 1-inch squares (2.54 cm^2) and sterilized by autoclaving before experimentation.

Fungal Spore Inoculation onto Materials

The fungi *Aspergillus niger*, *Stachybotrys chartarum*, *Penicillium chrysogenum*, and *Chaetomium globosum* were recovered from contaminated building materials and subcultured onto malt extract agar (MEA) plates.[2] These plates were incubated at 25°C until confluent growth was achieved, typically between 6–7 days. Spores were collected from these plates and inoculated into phosphate buffered saline (PBS), pH 7.0 at a rate of 240,000 spores/50 μL of PBS. The 50 μL PBS spore suspension was inoculated onto the materials and allowed to dry at room temperature before the experimental treatments of washing and steam cleaning were applied. This level of spores, if untreated, would result in confluent fungal growth on the material after 7 days of incubation.

Mycotoxin Inoculation onto Materials

Because trichothecene mycotoxins are produced by sick building syndrome-associated organisms such as *S. chartarum*,[3] in this trial, two macrocyclic trichothecenes (roridin A and verrucarin A) and one simple trichothecene (T-2) were used. The mycotoxins were inoculated at a level of 10 μg per square inch of material. This was achieved by suspending 200 μg of the mycotoxins into a 1.0 mL solution of 99.9% high-performance liquid chromatography (HPLC) grade methanol giving a final concentration of 10 μg/50 μL. The 50 μL was inoculated onto the materials and allowed to dry at room temperature before the experimental treatments were applied.

Experimental Treatments: Washing

The spore- and mycotoxin-inoculated materials were placed separately into sterile plastic tubes containing 15 mL of a 2% solution of a commercial detergent (Xtra; USA Detergents Inc., North Brunswick, N.J.) and a 2% solution of chlorine bleach (Ultra Clorox Regular; The Clorox Co., Oakland, Calif.) in deionized water. The temperature of the solution was 39°C. The tubes were agitated vigorously, three times per min for a total of 10 min. The tubes were then emptied of the solution and rinsed three times with deionized water for 5 min each in the same manner washing was performed. Positive and negative controls were included, that is, materials were inoculated but did not receive the experimental treatment, and materials received the experimental treatment but were not inoculated. This procedure was replicated six times.

Experimental Treatments: Steam Cleaning

A commercial "dry steam vapor" cleaner with a 3 L capacity was used (Eurosteam, Crowley, Texas). The cleaner was operated at a high-heat, low-moisture setting. The steam was directed onto the spore and mycotoxin inoculated materials from a distance of 6 inches for 10 sec. This procedure was replicated six times.

Experimental Treatments: Gamma Irradiation

Because the fungicidal activity of gamma irradiation is well established,[4,5] in this trial the main focus was its effects on mycotoxins; however, its effect on the spores of the selected organisms was also determined. Sterile cotton swabs were used to retrieve 2.54 cm^2 of growth of the four different fungi from confluent growth on MEA plates. In terms of mycotoxins, the inoculation technique was the same as used for the washing and steam cleaning techniques.

After the materials and swabs had been inoculated, they were transported to a commercial nuclear facility and irradiated at a dosage between 10–13 kiloGrays. Ten kiloGrays are equivalent to one megaRad. Positive and negative controls were employed, that is, materials and swabs were inoculated but did not receive an experimental treatment, and materials and swabs received the experimental treatment but were not inoculated. This process was replicated six times.

Determination of Treatment Efficacy: Fungal Spores

After the washing and steam cleaning treatments, the materials were retrieved from the tubes, dried, and placed in 10 mL of PBS. After 5 hours the samples were serially diluted using PBS at a 1 in 10 dilution rate three times. One hundred μL of each dilution was then added to potato dextrose agar (PDA) plates. After the gamma irradiation treatment, the inoculated swabs were directly plated onto PDA media.[2] All agar plates were incubated for 7 days at 25°C. After 7 days, colony forming units (CFU) of fungi were identified using macroscopic and microscopic morphology[1,6] and counted.

Determination of Treatment Efficacy: Mycotoxins, Toxin Extraction

After the washing, steam cleaning, and gamma irradiation treatments, all materials were suspended in 15 mL of 99.9% HPLC grade methanol and allowed to soak for 18 hours. The methanol was then poured into 20-mL glass scintillation vials. The crude toxin extract was allowed to dry to completion under a fume hood. The dried remnants were resuspended in 1 mL of 99.9% HPLC methanol and filtered through polyvinylidene fluoride membrane filters with a pore size of 0.22 microns. The resulting filtrates were then used for toxicity testing using a yeast toxicity assay.

Yeast Toxicity Assay

This assay is based on the procedure of Engler et al.[7] The principle of this assay is as follows: cultures of *Kluyveromyces marxianus* (No. 8554; American Type Culture Collection, Manassas, Va.) are incubated with the filtrates for 8 hours. The yeast culture is sensitive to the presence of trichothecene mycotoxins and will not grow in their presence even at very small quantities (100–250 nanograms/mL). Optical density (OD) readings are made every 2 hours. The assay is terminated at 8 hours. A high OD is a result of increased turbidity, which is a consequence of the growth of the organism. A low OD is due to little or no growth of the organism.

K. marxianus was grown at 37°C and stored at 4°C on yeast-peptone-glucose (YPG) agar. Cultures for inoculation of the assay were prepared by adding a single colony from an agar plate to 5 mL YPG-50 media in a culture tube. The tube was incubated in a rotary incubator for approximately 16 hours at 37°C to give the culture a final density of 1×10^8 cells/mL of YPG-50.

For the assay procedure, YPG-50 was supplemented with a stock solution of polymixin B sulfate (PMBS) (ICN Biomedicals, Aurora, Ohio) to give a final bioassay PMBS concentration of 15 mg/mL. Tests were run in triplicate. One hundred and thirty-six μL of PMBS-supplemented YPG-50 medium was added to the wells of a 96-well polystyrene microtiter plate. Eight μL of test sample or control was added to each well, followed by 16 μL of yeast inoculum to yield an initial cell density of approximately 1×10^8 cells/mL. Blank wells contained 152 μL of medium and 8 μL of water. Control wells consisted of 144 μL of medium and 16 μL of yeast inoculum. The plates were sealed and incubated on a plate shaker at 37°C for 8 hours (when cells reached stationary phase). Cell density was measured every 2 hours by measuring the absorbance in a microtiter plate reader at a wavelength of 550 nm. The absorbance was correlated with a *K. marxianus* 8-hour growth curve to determine cell density.

Statistical Analysis

With regard to fungal spores, mean CFU were determined from the plates inoculated with the treated materials and were compared using a Mann Whitney Rank Sum Test (SigmaStat, software, version 2.0; SPSS Inc., Chicago, Ill.) with the mean CFU from the plates inoculated with the untreated materials. In this analysis, because of the difference in efficacy between the two techniques, the washing and steam cleaning techniques were compared separately with the controls. With regard to mycotoxins, mean OD readings from the positive controls were compared with the mean OD readings from the gamma irradiated, washing, and steam treated materials using a one-way analysis of variance test. Conditions of normality and equal variance were met for this analysis.

RESULTS

Washing Technique: Fungal Spores

Table I shows the results for the washing technique for fungal spores. All spores except those of C. *globosum* were either inactivated or removed from the materials. Mean CFU of *C. globosum* were reduced on all items except paper ($p < 0.05$).

Washing Technique: Mycotoxins

Table II shows the results with regard to mycotoxin levels. The mean ODs of the cloth and paper filtrates were higher than the mean ODs of the filtrates of the positive controls ($p < 0.001$) and similar to the mean ODs of the negative controls, indicating that the filtrates were not toxic to *K. marxianus* cultures. The mean ODs of the carpet and wood filtrates were no different from the positive controls ($p > 0.05$) and lower than the mean ODs of the negative controls, indicating that the filtrates were toxic to the *K. marxianus* cultures.

TABLE I. The Effects of Washing with a Detergent/Bleach Combination on Fungal Spore Activity

		Washing Technique N = 9		Control N = 5		
Organism	**Material**	**Mean (CFU/2.54 cm^2)**	**SE**	**Mean (CFU/2.54 cm^2)**	**SE**	**p Value**
C. globosum	Wood	72*	19.3	636**	170	<0.01
	Cloth	293*	41.1	32446**	39077	<0.005
	Paper	290*	83.6	2720**	796.5	>0.05
	Carpet	272*	112.8	1396**	397.6	<0.05
S. chartarum	Wood	0	0	1300	748.3	NA
	Cloth	0	0	56	33.6	NA
	Paper	0	0	1328	2089	NA
	Carpet	0	0	1100	1043	NA
P. chrysogenum	Wood	0	0	8020	4346	NA
	Cloth	0	0	15800	4693	NA
	Paper	0	0	32600	5455	NA
	Carpet	0	0	5560	1689	NA
A. niger	Wood	0	0	8740	6328	NA
	Cloth	0	0	1420	542.6	NA
	Paper	0	0	8600	6853	NA
	Carpet	0	0	12236	991.8	NA

Notes: Controls received spores but no treatment. Different superscripts indicate differences at the level of significance given in the far column.
SE = standard error.
NA = not applicable.

TABLE II. Optical Density (Yeast Toxicity Assay for Mycotoxin Activity) Readings After Washing, Steam Cleaning, or Gamma Irradiation Treatment Techniques

		Positive Control		Washing		Steam		Gamma Irradiation	
Mycotoxin	**Material**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Roridin A	Wood	0.4*	0.05	0.26*	0.009	0.4A	0.05	0.14*	0.004
	Cloth	0.31*	0.016	1.24**	0.012	0.3A	0.019	0.12*	0.005
	Paper	0.27*	0.02	1.28**	0.009	0.3A	0.054	0.13*	0.003
	Carpet	0.25*	0.028	0.19*	0.014	0.3A	0.007	0.23*	0.01
T-2	Wood	0.46*	0.033	0.61*	0.03	0.51A	0.009	0.34*	0.05
	Cloth	0.3*	0.013	1.3**	0.034	0.32A	0.007	0.13*	0.005
	Paper	0.23*	0.021	1.29**	0.028	0.25A	0.02	0.16*	0.017
	Carpet	0.46*	0.034	0.39*	0.052	0.56A	0.023	0.29*	0.009
Verrucarin A	Wood	0.34*	0.006	0.3*	0.037	0.35A	0.011	0.16*	0.014
	Cloth	0.31*	0.005	1.31**	0.023	0.29A	0.011	0.12*	0.004
	Paper	0.27*	0.013	1.3**	0.027	0.27A	0.015	0.15*	0.01
	Carpet	0.36*	0.008	0.16*	0.012	0.32A	0.011	0.27*	0.017

Notes: N = Six per treatment. Positive controls were materials that had toxins added, but did not receive any treatment. Different superscripts indicate differences at the $p < 0.001$ level of significance.
SE = standard error.

Steam Cleaning Technique: Spores

Table III shows the results for the steam cleaning technique for fungal spores. Results were mixed, with no organisms being completely eliminated as with some of the washing results. Mean CFU of *P. chrysogenum* were reduced on all materials ($p < 0.05$). Mean CFU of *S. chartarum* were reduced on wood only ($p < 0.01$). Mean CFU of *C. globosum* were reduced on wood and carpet ($p < 0.05$). Mean CFU of *A. niger* were no different from the positive controls ($p > 0.05$).

Steam Cleaning Technique: Mycotoxins

Table II shows the results with regard to mycotoxin levels. The mean ODs of the filtrates from all treated materials were no different from the positive controls ($p > 0.05$) and lower than

TABLE III. Effects of a Steam Cleaning Technique on Spore Activity

		Steam Cleaning		Control	
Organism	**Material**	**Mean (CFU/2.54 cm)**	**SE**	**Mean (CFU/2.54 cm)**	**SE**
C. globosum	Wood	48*	19.4	636**	170
	Cloth	10950*	21040	32446*	39077
	Paper	933*	590.7	2720*	796.5
	Carpet	343*	331.5	1396**	397.6
S. chartarum	Wood	36*	59.9	1300*	748.3
	Cloth	50*	837	56*	33.6
	Paper	216*	162.5	1328*	2088.8
	Carpet	363*	498.1	1100*	1042.9
P. chrysogenum	Wood	321*	159.4	8020**	4346
	Cloth	166*	180.1	15800**	4692.9
	Paper	1255*	575.7	32600**	5455.3
	Carpet	1000*	816.5	5560**	1689.3
A. niger	Wood	1201*	782.6	8740*	6328.3
	Cloth	166*	73.6	1420*	542.6
	Paper	5516*	3206.3	8600*	6852.7
	Carpet	860*	647.5	12236*	9991.8

Notes: Different superscripts indicate a significant difference at the $p < .05$ level.
SE = standard error.

the negative controls, indicating that the filtrates were toxic to *K. marxianus* cultures.

Gamma Irradiation: Spores

The MEA plates from the inoculated swabs showed no growth after 7 days incubation at 25°C.

Gamma Irradiation: Mycotoxins

Table II shows the results with regard to mycotoxin levels. The mean ODs of the filtrates from all treated materials were no different from those of the positive controls ($p > 0.05$) and lower than the negative controls, indicating that the filtrates were toxic to *K. marxianus* cultures.

DISCUSSION

The results show that all three techniques had varying degrees of success on the tested materials, spores, and mycotoxins. With regard to the gamma irradiation results, many studies have been performed on gamma irradiation and mycotoxins, particularly mycotoxins on grains.[8–10] In these studies, results have been mixed with a range of levels of irradiation being required to inactivate different mycotoxins. Adjusting relative humidity during irradiation appears to play a role[11] although some studies have not shown any significant differences.[12] Possibly, the increase needs to be large for the treatment to become effective. In this study the technique was not successful with dry items, although other studies[5] have shown gamma irradiation to be effective in inactivating fungal spores and colonies. If mycotoxin inactivation is not a concern, then this technique appears to be quite effective, although attention must be given to the degrading effect of gamma irradiation on items.

With regard to the washing technique, the failure of the technique to inactivate *C. globosum* could be due to a number of factors, for example, the ascospores of *C. globosum* are retained inside a structure known as a perithecium[13] before release, and it is possible that the perithecium played a protective role in this situation. The bleach used in this trial contained sodium hypochlorite as the active agent. Sodium hypochlorite has been shown to have bactericidal properties.[14] It is also fungicidal to certain organisms.[15] One study showed that it had mixed results at 20°C against *Penicillium roqueforti*.[16]

In terms of toxins, 20 mL of a 1.3% concentration of sodium hypochlorite is sufficient to inactivate 20 μg of pure aflatoxin.[17] However, this technique has a proviso that 5% acetone is used as a rinse afterward to either remove or inactivate a carcinogenic byproduct, 2,3-dichloro aflatoxin B_1, which is formed out of the inactivation process. Sodium hypochlorite has also been used to inactivate trichothecene mycotoxins at a concentration between 3 and 5%.[18] In this trial it was successfully used in conjunction with a commercial detergent on paper and cloth. The failure of this treatment to inactivate mycotoxins on carpet and wood could be because the carpet fibers presented an effective physical barrier to the detergent/bleach, and the wood absorbed the alcohol-based mycotoxin preparations. Both features could prevent the washing treatment from accessing the mycotoxins.

With regard to the steam cleaning technique, it was not as effective as the washing technique but was successful in significantly reducing C. *globosum* and S. *chartarum* spore burdens on wood. The technique involved a 10-sec spraying time. It is conceivable that longer time periods could result in effective removal of spore burdens. Also, using a biocide in the steam cleaner may provide an effective treatment.

In this study the focus was on the effects of the treatments on fungal spores and mycotoxins. However, a recent study[19] showed that fungal colonies can aerosolize large numbers of antigenic fungal fragments into the environment simultaneously with fungal spores. These fragments were shown to be much smaller than fungal spores. It is possible that these fragments also carry mycotoxins and contribute to fungal contamination of contents. The cleaning techniques tested in this trial may be effective against fungal fragments but this remains to be examined.

While the techniques described have shown varying degrees of success in inactivating mycotoxins and fungal culturability, it must be noted that fungal spores can still have allergenic properties even when they are nonviable. High efficiency particulate air [filter] vacuuming is often used in mold remediation situations and may have application with regard to the removal of spores from contents.

CONCLUSION

In this trial, gamma irradiation at a 10–13 Kgy exposure was successful in inactivating spores of *Aspergillus niger, Stachybotrys chartarum, Penicillium chrysogenum,* and *Chaetomium globosum*, but not the mycotoxins roridin A, verrucarin A, and T-2 that had been inoculated onto carpet, cloth, paper, and wood. Washing with bleach and a commercial detergent was effective against spores of *A. niger, S. chartarum,* and *P. chrysogenum*, but not *C. globosum*. The treatment was successful against the tested mycotoxins for cloth and paper materials, but not carpet and wood. The steam cleaning technique was successful only with spores of *S. chartarum* and *C. globosum* on wood material.

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